Rovedar

JWPS

Journal of World's Poultry Science. 2024; 3(1): 1-11.

DOI: 10.58803/jwps.v3i1.25

http://jwps.rovedar.com/

Research Article

The Effects of *Allium sativum* on Growth Performance, Kidney and Liver Function Markers, Microbial Flora and Feed Digestibility in Broiler Chickens

Tchantchou Chamberlin Djamen[1,*], Kondo Camile Nyembo[2], Tadjong Ruben Ngouana[1], Deffo Gilchrist Tchouan[3], Mikael Donfack[1], Tagne Achille Bernard Fokam[1], and Jean Raphaël Kana[1]

[1]Animal Production and Nutrition Research Unit, Department of Animal Science, Faculty of Agronomy and Agricultural Sciences, University of Dschang, Cameroon

[2]Department of Animal Sciences and Plant Technology, Faculty of Agronomic Sciences, La Sapientia Catholic University of Goma, Democratic Republic of Congo

[3]Institut Universitaire Evangélique du Cameroun, Faculté d'Agronomie et des Sciences de l'Environnement, Unité de recherche et d'Application en Production Animale, BP 27 Bandjoun Cameroun

*** *Corresponding author:*** Djamen Tchantchou Chamberlin, Department of Animal Science, Faculty of Agronomy and Agricultural Sciences, University of Dschang, Cameroon. Email: djamenchamberlin@gmail.com

ARTICLE INFO

Article History:
Received: 02/01/2024
Revised: 01/02/2024
Accepted: 19/02/2024
Published: 25/03/2024

Keywords:
Allium sativum
Broiler chicken
Digestibility
Growth performance
Gut microbiota

ABSTRACT

Introduction: The global ban on antibiotics as a feed additive, prompted by concerns over microbial resistance and the accumulation of antibiotic residues in animal products, has urged researchers to explore natural alternatives. These alternatives modulate the gut microbiota and enhance animal production performance. This study aimed to assess the impact of *Allium sativum* on the production performance of broiler chickens.

Materials and methods: A total of 280 day-old broiler chicks were examined for 42 days. After being sexed, they were randomly allocated into five groups, each consisting of 14 chicks, with four replications. The control group was fed on a ration without additives (R0-). The treatment groups consisted of a control diet supplemented with 1 g of antibiotic Doxycycline® per kg of basal diet and per liter of drinking water (R0+) and 5 g garlic per kg of basal feed and also per liter of drinking water (RAs), respectively. To facilitate the oral administration, the solution was absorbed by charcoal from the fruit stones of *Canarium schweinfurthii* at a rate of 100 g and 100 ml per kg of diet and per liter of drinking water, respectively. The investigated parameters included growth performance, microbial flora, markers of kidney, and liver function, and feed digestibility.

Results: The results revealed *Allium sativum* significantly decreased feed intake, feed conversion ratio, serum aspartate aminotransferase, alanine aminotransferase, and urea levels regardless of the administration mode in broiler chickens, compared to the negative control. When administered through both feed and drinking water, garlic significantly increased live weight, weight gain, and lactic acid bacteria count, compared to the negative control. Feeding garlic to broilers had insignificantly affected the digestibility of feed components irrespective of the administration mode.

Conclusion: In conclusion, *Allium sativum* can effectively serve as a feed additive in broiler diets or drinking water, promoting growth performance without harming kidney and liver functions. The present findings help address concerns about antibiotic resistance and residues in poultry products.

1. Introduction

Microbial resistance and the accumulation of antibiotic residues in animal products have led to a worldwide ban on antibiotics as feed additive[1,2]. Faced with this situation, researchers have turned to natural alternatives capable of modulating the gut microbiota and improving animal production performance. These alternatives include

► *Cite this paper as*: Djamen CT, Nyembo KC, Ngouana TR, Tchouan DG, Donfack M, Fokam TAB, Kana JR. The Effects of *Allium sativum* on Growth Performance, Kidney and Liver Function Markers, Microbial Flora and Feed Digestibility in Broiler Chickens. Journal of World's Poultry Science. 2024; 3(1): 1-11. DOI: 10.58803/jwps.v3i1.25

products derived from plants, commonly known as phytobiotics, which have bioactive compounds that positively affect the growth performance of animal[3,4]. These feed additives include a wide range of dietary herbs and spices, which contain a wide variety of volatile and non-volatile chemical substances with aromatic, medicinal, or nutritional properties[3,5,6]. Garlic (*Allium sativum*) is a plant with extremely varied therapeutic and nutritional properties [7,8]. Studies have identified the presence of active substances that positively impact animal performance[9]. These substances include saponins, terpenoids, anthocyanins, flavonoids, tannins and phenols[10], possessing antibacterial[11,12], anti-fungal[13,14], antioxidant[15,16] and antiviral properties[8]. Rinkesh et al.[17] reported that 0.1% garlic has a beneficial effect on the growth performance, carcass yield and production cost of broiler chickens. In the same line, Franciszek et al.[18] reported that 2.25 mg garlic extract/kg feed increased feed intake, body weight and carcass yield in chickens at 42 days. The positive effect of garlic on increasing body weight in chickens may result from feed digestibility improvement and the modulation of the gut microbial composition. Garlic and garlic products have shown a broad antibiotic spectrum against gram-positive and gram-negative bacteria and are effective against many common pathogens and intestinal bacteria responsible for diarrhoea in humans and animals[19]. Chiang et al.[20] showed the stimulatory effect of organosulfur compounds in garlic oil on nitric oxide and prostaglandin E2 in stimulated macrophages, which may be associated with the antibacterial activity of aqueous garlic extract. In poultry, phytobiotics are generally incorporated into the feed, but they can also be incorporated into the drinking water. Although garlic has already been the subject of numerous studies in animal feed, the results obtained is always inferior to those of antibiotics, probably due to the loose of the active compounds during processing. These compounds could be better preserved if administered to the animals as supplementation. This hypothesis was the main reason for the present study, with the main objective to assess the effects of oral administration of *Allium sativum* on the digestibility of feed components and production performance of broiler chickens.

2. Materials and Methods

2.1. Ethical approval

This study was carried out in strict accordance with the recommendations of institutional guidelines for the care and use of laboratory animals. Chickens were humanly handled with respect to the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

2.2. Study area

This study occurred at the Teaching and Research Farm (TRF) of the University of Dschang, Cameroon between January and February 2022. The TRF is located between 05°26' and 5°44' north latitude, and 9°94 and 10°06 east longitude, at an altitude of 1,420 m above sea level. The rainy season lasts from mid-September to mid-November and a dry season for the rest of the year. Rainfall varies between 1,500 and 2,000 mm per year. The average temperature is around 21°C and the average relative humidity is 76.8%.

2.3. Feed additive

Allium sativum bulbs were purchased at the Dschang market, Western Cameroon. The garlic was washed, crushed using a Moulinex (China), and then soaked in a hermetically sealed bucket for 3 days for fermentation in the proportions of 500g of garlic/liter of water. After fermentation, the resulting solution was filtered through Wattman paper (3 MM). The filtrate recovered was administered to the chickens through drinking water and feed. A preliminary test on 100 broilers was carried out to determine the quantity of solution to be administered per liter of drinking water, and the dose of 5 ml of garlic per liter of water was adopted.

In order to facilitate the oral administration of the garlic into the feed, 100 ml of the material was absorbed by 100g of charcoal from *Canarium schweinfurthii* seeds. The solution absorbed in the charcoal was evaporated in a ventilated oven at 50°C for 24 hours and the charcoal containing the garlic extract was incorporated in the chicken feed at a rate of 5g of charcoal/kg of feed[21].

2.4. Animals

A total of 280 day-old Cobb500 strain chicks were sexed and randomly divided into 5 groups with 4 replicates of 14 chicks each, including 7 males and 7 females. They were reared on litter at a density of 20 chicks/m^2 at the brooding phase and 10 chicks/m^2 at the finishing phase. Feed and water were provided *ad libitum* throughout the trial.

2.5. Prophylaxis

The chicks were vaccinated against infectious bronchitis (H120® Holland) and Newcastle disease (Hitchner B1® Holland) on day 7 with a booster on day 18 and against Gumboro disease (IBA Gumboro® Holland) on day 10. An anti-stress (5 g in 2 liters of water, INTROVITA+WS, Holland) agent was added to the drinking water for the first 3 days, as soon as the chicks entered the brooder and each time before and after vaccination and weighing of the chicks. A foot bath was placed at the entrance to each rearing house and the disinfectant consisted of bleach and cresyl, which was renewed every 3 days.

2.6. Experimental diets

Chickens in the control group were fed on ration ($R0^-$) without additives (Table 1). The other treatment groups consisted of a control diet supplemented with 1 g of antibiotic Doxycycline® per kilogram of basal diet and per liter of drinking water (R0+) and 5 g garlic per kilogram of basal feed and also per liter of drinking water (RAs), respectively.

Table 1. Composition of experimental diets at the starter and finisher phases for 42 days of the study

Ingredients (% Dry matter)	Starter Phase (1-21 days)	Grower Phase (22-42 days)
Maize	60	67
Cotton seed cake	5	5
Soya bean meal 49	22	15
Fish meal	5	5
Wheat bran	2	2
Shell	1	1
Premix 5%*	5	5
Total	100	100
Analyzed chemical composition		
Crude cellulose (% DM)	3.15	3.25
Calculated chemical composition		
Metabolizable energy (kcal/kg)	2977	3108
Crude protein (%)	23.01	20.3
Energy /protein	129.4	153.1
Calcium (%)	1.05	1.03
Phosphorus (%)	0.6	0.6
Calcium/Phosphorus	1.75	1.72
Lysine (%)	1.4	1.2
Methionine (%)	0.5	0.45
Lysine/Methionine	2.8	2.7
Cellulose (%)	2.43	2.61

*Premix 5%: Crude protein: 40%, Calcium:8%, Phosphorus:2.05%, Lysine: 3.3%, Methionine: 2.40%, Metabolizable energy: 2078 kcal/k, Vitamin A: 3,000,000 IU, Vitamin D3 600,000 IU, Vitamin E: 4,000 mg, Vitamin K: 500 mg, Vitamin B1: 200 mg, Vitamin B2: 1000 mg, Vitamin B6: 4000 mg, Vitamin B12: 4 mg, Iron: 8000 mg, Cu: 2000 mg, Zn: 10,000 mg, Se: 20 mg, Mn: 14,000 mg, DM: Dry matter, ME: Metabolizable energy

2.7. Production performance and biochemical markers

Throughout the study period (1-42 days), growth parameters (feed intake, water consumption, weight gain, live weight, and feed conversion ratio (FCR) were collected weekly. At the end of the study, 5 female and 5 male chickens were randomly selected from each group. They were then fasted for 24 hours to evacuate all digestive tract contents, weighed, plucked, and eviscerated without anesthesia. Carcass yield and relative weights of organs were calculated. Moreover, intestine length was measured using a measuring tape, and intestine density was calculated by dividing the intestine length by the intestine weight.

$$\text{FCR} = \frac{\text{Amount of feed consumed (g)}}{\text{Weight gain (g)}}$$

In the next step, 5 ml of blood samples from jugular vein were collected in tubes without anticoagulant was used to measure alanine aminotransferase (ALT), aspartate aminotransferase (AST), Urea, Creatinine, Triglyceride, Total cholesterol, high-density lipoproteins (HDL) and low-density lipoproteins (LDL) Cholesterol according to kit manufacturers' instructions (Chronolab®, Barcelona, Spain).

2.8. Gut microbiota

At the end of the trial (42 days), fecal samples were collected from the cloaca of four chickens per treatment (two males and two females), using cloacal swabs and immediately used for the identification and quantification of lactic acid bacteria, *E. coli* and Salmonella in their respective specific culture media, for determining of Lactic acid bacteria, the culture medium used was lactobacilli M.R.S AGAR produced by Acumedia® (India) and ISO 9001 reference. The final pH was 7.5 ± 0.2 at 25°C. The preparation procedure consisted of dissolving 70 g of this medium in 1 liter of distilled water in an Erlenmeyer flask, then heating with frequent stirring until complete dissolution. This medium was autoclaved at 121°C for 15 minutes. For *E. coli*, the culture medium used was Mac Conkey Agar manufactured by Liofilchem® (India, diagnostic and reference ISO 610028). The final pH was 7.1 ± 0.2 at 25°C. The preparation procedure consisted of pouring 51.5 g of the suspension into 1 liter of distilled water, and then heating the mixture until completely dissolved. Finally, it was autoclaved at 121°C for 15 minutes. For *Salmonella*, the culture medium used was SS AGAR of reference ISO 610042 and produced by Liofilchem® (India) diagnostic. The final pH was 7 ± 0.2 at 25°C. The preparation procedure consisted of pouring 52 g of the suspension into 1 liter of distilled water, then boiling until complete dissolution without autoclaving according to the manufacturer's prescription. The inoculum was prepared by decimal dilutions, which consisted of placing 9 ml of physiological water in tubes numbered at the base by the type of sample and the dilution number. The swab bearing the sample was then introduced into the first tube. The latter was shaken to homogenize the solution (S1), then 1 ml of S1 was taken with a micropipette and introduced into the second tube to complete the solution to 10 ml, thus obtaining the 10-2 dilution. After homogenizing this solution, the procedure was carried out up to the 10-8 dilution. 1 ml of the 10-6 and 10-8 dilutions of each sample was taken and introduced into a petri dish each [22]. The previously prepared solution of each culture medium (MRS Agar, SS Agar, and Mac Conkey Agar) was introduced each time just after the introduction of the inoculum into the petri dish and homogenized.

2.9. Digestibility of feed components

The apparent digestive utilization coefficients (aDUC) of feed components were evaluated for 6 broiler chickens, including 3 males and 3 females, per treatment for 3 consecutive days. The 6 chicks per treatment were kept in the digestibility cages, and tarps were placed underneath the cages after 3 days of the adaptation period to collect faces from each replicate. Feed was weighed before feeding. Afterward, faces and feed refusals were collected and weighed daily for 3 days. Fecal samples were oven-dried at 60°C to constant weight for proximate analysis of DM and organic matter (OM) following AOAC processes[23]. Neutral Detergent Fiber (NDF) was determined by Van Soest et al. [24] method, and crude protein (CP) by the Kjedhal method. The apparent digestive utilization coefficients (aDUC) of DM, OM, CP, and NDF of the experimental diets were calculated.

2.10. Statistical analysis

The statistical software Statistical Package for Social

Table 2. Feed and water intake in broiler chickens fed with *Allium sativum* during 42 days of the experiment

Period (days)	Diets				
	Mode of administration	R0⁻	R0⁺	RAs	p-value
Feed intake (g)					
1-21	Feed	1338.54± 9.25[a]	1249.69±28.40[b]	1204.59±52.81[b]	0.011
	Water		1247.52±79.25[ab]	1162.80±13.25[b]	0.012
	p-value		0.262	0.093	
22-42	Feed	3659.88±202.44[a]	3131.63±39.55[b]	3073.05±140.37[b]	0.005
	Water		3557.79±190.38[a]	2936.97±133.07[b]	0.005
	p-value		0.097	0.810	
1-42	Feed	4998.43±216.15[a]	4381.32±66.80[b]	4277.64±192.99[b]	0.004
	Water		4805.32±115.14[a]	4099.78±142.45[b]	0.001
	p-value		0.264	0.504	
Water intake (ml)					
1-21	Feed	2975.31±55.37	2870.14±20.22	2939.43±107.33	0.258
	Water		2812.44±142.01	2771.07±79.25	0.095
	p-value		0.211	0.425	
22-42	Feed	8366.15±222.44	8365.86±649.78	8421.38±283.75	0.992
	Water		7925.90±241.04	7872.79±260.79	0.088
	p-value		0.096	0.616	
1-42	Feed	11341.46±272.87[a]	11236.00±633.51	11360.81±824.55	0.966
	Water		10738.34±99.24[b]	10643.86±334.23[b]	0.031
	p-value		0.179	0.269	

[a, b] Means same letters on the same line are not significantly different ($p > 0.05$); RAs: Diet supplemented with 5g of *Alium sativum* via feed and water; Group 1: R0-: Ration without additives (negative control), Group 2: R0+: 1g Doxycycline®/kg feed, Group 3: R0+: 1g Doxycycline®/liter water, Group 4: 5 g *Allium sativum*/kg feed, Group 5: 5g *Allium sativum*/liter of water

Sciences (SPSS version 20.0) was used for the analyses. All collected data were submitted to a one-way analysis of variance (ANOVA). Duncan's multiple range test was used to separate significant levels at $p < 0.05$. The normality of data was tested by the Shapiro-Wilk test.

3. Results

3.1. Chicken feed and water consumption

Regardless of the study period, chicken feed intake decreased significantly with the oral administration of garlic in the feed and drinking water, compared to the negative control ($p < 0.05$, Table 2). The decrease in feed and water intake was observed to be 11.12% and 15.11% during the starter phase (1-21 days), 19.09% and 24.65% during the finisher phase (22-42 days), and 16.85% and 21.92% over the entire study period (1-42 days), respectively.

3.2. Growth performance

At the starter phase and throughout the trial period, the live weight and weight gain of chickens orally administered garlic via feed or drinking water increased significantly compared to the negative control ratio ($p < 0.05$, Table 3). When comparing the administration modes across all study periods, no significant difference was recorded among the treatment groups for live weight and weight gain, except in the starter phase. In this phase, the incorporation of the antibiotic into the drinking water induced an increase in the live weight of the

Table 3. Effects of different treatments on live weight and weight gain in broilers chickens over a 42-day study period

Period (days)	Diets				
	Mode of administration	R0⁻	R0⁺	RAs	p-value
Live weight (g)					
1-21	Feed	612.71±11.06[b]	718.93±3.21[aB]	746.95±35.89[a]	0.001
	Water		767.07±40.65[aA]	794.95±6.15[a]	0.002
	p-value		0.021	0.738	
22-42	Feed	2029.98±14.64[b]	2245.78±106.51[a]	2250.07±79.49[a]	0.020
	Water		2308.46±27.78[a]	2319.31±91.18[a]	0.004
	p-value		0.099	0.625	
Weight gain (g)					
1-21	Feed	571.32±11.06[b]	691.34±20.75[a]	719.36±38.00[a]	0.001
	Water		739.48±56.79[a]	710.03±27.10[a]	0.003
	p-value		0.211	0.425	
22-42	Feed	1417.26±3.58	1526.85±108.41	1503.10±293.89	0.306
	Water		1541.39±24.30	1552.69±119.36	0.102
	p-value		0.096	0.616	
1-42	Feed	1988.59±14.64[b]	2218.18±124.96[a]	2222.48±59.01[a]	0.018
	Water		2280.87±35.14[a]	2262.71±135.72[a]	0.008
	p-value		0.179	0.269	

[a, b]Means same letters on the same line are not significantly different ($p > 0,05$). [A, B]: Means same letters in the same column are not significantly different ($p > 0.05$); RAs: Diet supplemented with 5g of *Alium sativum* via feed and water; Group 1: R0-: Ration without additives (negative control), Group 2: R0+: 1g Doxycycline®/kg feed, Group 3: R0+: 1g Doxycycline®/liter water, Group 4: 5 g *Allium sativum*/kg feed, Group 5: 5g *Allium sativum*/liter of water

Table 4. Feed conversion rate in broiler chickens fed with *Allium sativum* during 42 days of the study

Period (days)	Diets				
	Mode of administration	R0-	R0+	RAs	p-value
Feed conversion ratio					
1-21	Feed	2.34±0.08[a]	1.81±0.07[b]	1.68±0.16[b]	0.001
	Water		1.70±0.20[b]	1.64±0.07[b]	0.001
	p-value		0.111	0.120	
22-42	Feed	2.58±0.14[a]	2.06±0.13[b]	2.05±0.03[b]	0.002
	Water		2.31±0.15[a]	1.90±0.18[b]	0.006
	p-value		0.895	0.071	
1-42	Feed	2.51±0.12[a]	1.98±0.09[b]	1.92±0.04[b]	0.000
	Water		2.11±0.07[b]	1.82±0.14[c]	0.001
	p-value		0.587	0.062	

[a, b, c]: Means bearing the same letter on the line are not significantly different (p>0.05); RAs: Diet supplemented with 5g of *Alium sativum* via feed and water; Group 1: R0-: Ration without additives (negative control), Group 2: R0+: 1g Doxycycline®/kg feed, Group 3: R0+: 1g Doxycycline®/liter water, Group 4: 5 g *Allium sativum*/kg feed, Group 5: 5g *Allium sativum*/liter of water

chicks by 6.28%, compared to the batch that received the antibiotic in the feed (p > 0.05).

3.3. Feed conversion ratio

The incorporation of *Allium sativum*, irrespective of the mode of administration and the study phase, induced significant decreases in the feed conversion ratio compared to the negative control (p < 0.05, Table 4). With the incorporation of this phytobiotic in the feed, FCR decreases by about 39.29% at the starter phase, 25.85% at the finisher phase (22-42 days), and 30.73% over the entire study period. Via drinking water, *Allium sativum* induced decreases in feed conversion ratio for about 42.68%, 35.79%, and 37.91% respectively at the starter phase, finisher phase, and over the entire study period.

3.4. Carcass and digestive organ characteristics

The incorporation of *Allium sativum* into the feed and drinking water did not significantly affect the carcass characteristics, relative organ weights, and digestive organ measurements of broiler chickens (p> 0.05, Tables 5 and 6).

3.5. Gut microbiota

Whatever the mode of administration, garlic has no significant effect on lactobacillus and *salmonella* counts (p > 0.05). However, although comparable to the negative control, the phytobiotic tends to increase the number of lactobacilli in the digestive tract of broilers. *Allium sativum* incorporation as a feed additive reduced the number of *Escherichia coli* by 6.41% compared to the negative control (p < 0.05, Table 7).

3.6. Liver function and renal function markers

Supplementing broilers with garlic via water and feed induced a significant decrease in the serum AST and ALT content compared to the negative control (p < 0.05). However, this additive had no significant effect on serum protein content regardless of the mode of administration (p > 0.05).

Table 5. Effects of *Allium sativum* on carcass characteristics of broiler chickens during 42 days of the study

Characteristics (% LW)	Diets				
	Mode of administration	R0-	R0+	RAs	p-value
Carcasse yield	Feed	72.94± 2.99	73.51±1.49	73.22±1.3	0.254
	Water		73.56± 2.53	73.82± 0.64	0.780
	p-value		0.097	0.133	
Head	Feed	2.23±0.27	2.34±0.22	2.36±0.15	0.652
	Water		2.25±0.17	2.22±0.14	0.902
	p-value		0.317	0.873	
Legs	Feed	3.77± 0.26	3.95± 0.42	4.03± 0.41	0.396
	Water		3.94± 0.53	3.97± 0.55	0.698
	p-value		0.459	0.237	
Liver	Feed	1.83±0.26	1.73± 0.11	1.77± 0.15	0.777
	Water		1.81±0.17	1.88±0.13	0.217
	p-value		0.278	0.698	
Heart	Feed	0.53± 0.09	0.52±0.05	0.54±0.09	0.743
	Water		0.53± 0.07	0.51± 0.07	0.780
	p-value		0.442	0.783	
Abdominale fat	Feed	1.19± 0.29	1.13± 0.46	0.82± 0.27	0.215
	Water		1.09± 0.27	0.88± 0.22	0.465
	p-value		0.115	0.762	

RAs: Diet supplemented with 5g of *Alium sativum* via feed and water; Group 1: R0-: Ration without additives (negative control), Group 2: R0+: 1g Doxycycline®/kg feed, Group 3: R0+: 1g Doxycycline®/liter water, Group 4: 5 g *Allium sativum*/kg feed, Group 5: 5g *Allium sativum*/liter of water, LW: Live weight

Table 6. Measurements of digestive organs according to the mode of administration of *Allium sativum* during 42 days of the study

Characteristics (% LW)	Diets				
	Mode of administration	R0⁻	R0⁺	RAs	p-value
Gizzard weight (% LW)	Feed	1.60±0.23	1.52±0.13	1.70±0.20	0.076
	Water		1.74±0.07	1.61±0.15	0.295
	p-value		0.143	0.178	
Pancreas weight (% LW)	Feed	0.26±0.04	0.21±0.05	0.24±0.04	0.476
	Water		0.22±0.07	0.21±0.04	0.465
	p-value		0.922	0.639	
Length of intestine (cm)	Feed	183,73± 43,17	182,58± 20,63	184,58± 35,77	0,887
	Water		199,20±19,75	188,08± 28,09	0,902
	p-value		0.077	0.966	
Weight of intestine (g)	Feed	100,36± 35,93	95,92± 12,57	94,80±23,38	0,987
	Water		94,42± 17,05	94,67± 11,73	0,932
	p-value		0.077	0.726	
Density of intestine (g/cm)	Feed	0,56± 0,16	0,53± 0,06	0,54± 0,20	0,864
	Water		0,47± 0,09	0,51± 0,10	0,765
	p-value		0.290	0.174	

PV: live weight; RAs: Diet supplemented with 5g of *Alium sativum* via feed and water; Group 1: R0-: Ration without additives (negative control), Group 2: R0+: 1g Doxycycline®/kg feed, Group 3: R0+: 1g Doxycycline®/liter water, Group 4: 5 g *Allium sativum*/kg feed, Group 5: 5g *Allium sativum*/liter of water; LW: Live weight

Administration of garlic through drinking water to broilers induced a significant decrease in serum urea content compared to the negative control ($p < 0.05$). In the same line, supplementing broiler feed with *Allium sativum* significantly lowered the serum creatinine level compared to the negative control ($p < 0.05$, table 8).

Table 7. Gut microbiota count in broiler chickens according to different routes of administration of *Allium sativum* during 42 days of study

Bacteria count (Log 10 CFU)	Diets				
	Mode of administration	R0⁻	R0⁺	RAs	p-value
Lactobacilli	Feed	9.42±0.61	9.72±0.03	11.78±0.71	0.212
	Water		9.76±0.15	12.01±0.61	0.128
	p-value		0.075	0.956	
Escherichia coli	Feed	4.98±0.46[a]	5.39±0.09[a]	4.68±0.33[b]	0.014
	Water		5.44±0.20	4.33±0.58	0.291
	p-value		0.700	0.880	
Salmonella	Feed	5.95±0.01	5.90±0.09	5.75±0.05	0.103
	Water		5.98±0.07	6.02±0.0	0.058
	p-value		0.058	0.216	

[a, b] Means same letters on the line are not significantly different ($p > 0.05$); RAs: Diet supplemented with 5g of *Alium sativum* via feed and water; Group 1: R0-: Ration without additives (negative control), Group 2: R0+: 1g Doxycycline®/kg feed, Group 3: R0+: 1g Doxycycline®/liter water, Group 4: 5 g *Allium sativum*/kg feed, Group 5: 5g *Allium sativum*/liter of water; UFC: Colony forming unit per liter of water

Table 8. Effects of different treatments on liver and kidney function markers in broiler chickens during 42 days of study

Parameters	Diets				
	Mode of administration	R0⁻	R0⁺	RAs	p-value
Markers of liver function					
AST (UI/L)	Feed	144.05±16.84[a]	89.19±13.52[b]	97.25±7.40[bB]	0.000
	Water		148.76±27.35[a]	102.49±17.49[bA]	0.009
	p-value		0.295	0.038	
ALT(UI/L)	Feed	132.17±27.34[a]	84.68±16.85[b]	90.44±10.58[b]	0.007
	Water	132.17±27.34[b]	167.09±6.25[a]	100.83±7.07[c]	0.000
	p-value		0.182	0.401	
Total protein (g/dL)	Feed	4.35±0.22	4.35±0.18	4.44±0.17	0.563
	Water		4.36±0.13	4.31±0.13	0.799
	p-value		0.342	0.394	
Markers of renal function					
Creatinine(mg/dL)	Feed	3.10±0.74[b]	2.76±0.76[b]	4.43±0.60[a]	0.003
	Water		2.65±0.68	2.71±0.32	0.432
	p-value		0.288	0.198	
Urea (mg/dL)	Feed	19.52±0.44	19.65±0.53	19.57±0.42	0.861
	Water	19.52±0.44[a]	19.29±0.57[ab]	18.84±0.41[b]	0.046
	p-value		0.951	0.611	

[a, b, c]: means same letters in the same row are not significantly different ($p>0.05$); [A, B]: means same letters in the same column are not significantly different ($p>0.05$); RAs: Diet supplemented with 5g of *Alium sativum* via feed and water; Group 1: R0-: Ration without additives (negative control), Group 2: R0+: 1g Doxycycline®/kg feed, Group 3: R0+: 1g Doxycycline®/liter water, Group 4: 5 g *Allium sativum*/kg feed, Group 5: 5g *Allium sativum*/liter of water; AST: Aspartate amino transferase, ALT: Alanine amino transferase

Table 9. Evaluation of cholesterol and triglyceride in broiler chickens fed *Allium sativum* via different routes during 42 days of study

Parameters	Diets				
	Mode of administration	R0-	R0+	RAs	p-value
Triglyceride (UI/L)	Feed	107.20±5.14	107.14±4.56	104.21±5.19	0.403
	Water		109.06±7.19	107.55±4.22	0.807
	p-value		0.109	0.645	
Total cholesterol (UI/L)	Feed	161.64±6.20^{b}	179.65±7.61^{a}	155.56±5.34^{bA}	0.000
	Water	161.64±6.20^{a}	146.74±10.50^{b}	155.04±10.84^{aB}	0.034
	p-value		0.542	0.042	
Cholesterol HDL (UI/L)	Feed	33.07±4.07^{b}	51.17±10.88^{a}	32.36±5.10^{b}	0.001
	Water		29.19±5.72	30.51±5.08	0.434
	p-value		0.373	0.772	
Cholesterol LDL (UI/L)	Feed	104.77±9.85	108.34±6.61	104.26±3.05	0.284
	Water		102.02±9.08	104.57±3.36	0.586
	p-value		0.303	0.693	

$^{a, b}$: means same letters in the same row are not significantly different (p>0.05); $^{A, B}$: means same letters in the same column are not significantly different (p > 0.05); RAs: Diet supplemented with 5g of *Alium sativum* via feed and water; Group 1: R0-: Ration without additives (negative control), Group 2: R0+: 1g Doxycycline®/kg feed, Group 3: R0+: 1g Doxycycline®/liter water, Group 4: 5 g *Allium sativum*/kg feed, Group 5: 5g *Allium sativum*/liter of water

3.7. Total cholesterol and triglycerides

Oral administration of garlic as a phyto additive had no significant effect on serum triglyceride and LDL-cholesterol content in broilers (p > 0.05). However, serum content in total cholesterol and HDL-cholesterol decreased significantly with the garlic administration via feed, compared to the group of broilers fed on antibiotics via feed (p < 0.05). Via drinking water; this phyto-additive significantly increased (p < 0.05) the serum content of Total cholesterol compared to the group of broilers supplemented with antibiotics via drinking water (Table 9).

3.8. Digestibility of feed components

Regardless of the mode of administration, garlic incorporation did not significantly affect the digestibility of feed components (p > 0.05). Although not statistically significant, the digestibility of dry matter (DM), organic matter (OM), and crude protein (CP) showed an increase with the administration of this phyto-additive to broilers via both feed and drinking water compared to the negative control (Table 10).

Table 10. Effects of the method of administration of *Allium sativum* on the digestibility of food constituents

aDUC (%)	Diets				
	Method of administration	R0-	R0+	R As	p-value
aDUC Dry matter	Feed	81.50±0.65	84.82±1.84	82.23±1.84	0.086
	Water		81.36±2.89	83.69±2.97	0.463
	p-value		0.634	0.301	
aDUC Organic matter	Feed	86.51±0.39	88.38±1.07	86.83±1.75	0.209
	Water		86.53±1.07	87.68±1.75	0.639
	p-value		0.532	0.612	
aDUC Crude protein	Feed	93.44±0.38	94.32±0.67	93.09±0.73	0.113
	Water		93.78±0.23	94.32±1.11	0.350
	p-value		0.187	0.640	
aDUC NDF	Feed	79.90±2.95	77.30±1.61	73.78±6.39	0.272
	Water		81.18±3.16	83.48±3.95	0.468
	p-value		0.408	0.334	

RAs: Diet supplemented with 5g of *Alium sativum* via feed and water; Group 1: R0-: Ration without additives (negative control), Group 2: R0+: 1g Doxycycline®/kg feed, Group 3: R0+: 1g Doxycycline®/liter water, Group 4: 5 g *Allium sativum*/kg feed, Group 5: 5g *Allium sativum*/liter of water; aDUC: Apparent digestive utilization coefficient; DM: dry matter; OM: organic matter; NDF: Neutral detergent fiber; CUDa: coefficient of apparent digestive utilization PB: Crude protein

4. Discussion

Allium sativum supplementation induced a significant decrease in feed and water intake in broilers, irrespective of the mode of administration and the study period. This low feed intake could be due to allicin, an organosulphur responsible for garlic's repellent taste [25]. This result is similar to those of Jimoh et al [26] who reported that garlic reduces feed intake without degrading the feed conversion ratio in chickens. The present results are contrary to the findings of ManSoub and Myandoab [27], who reported that incorporating garlic into the feed at low doses (0.125 and 0.25%) significantly increased feed intake in chickens. Similarly, Elagib et al.[28] showed that 3% garlic powder increased feed intake by 27% and feed efficiency by 22%. The decrease in water intake recorded in this study corroborates the findings of Necdem et al.[29] who reported that the incorporation of *Pentadiplandra brazzeana* powder at increasing rates in the drinking water of broilers resulted in a linear decrease (p < 0.05) in water intake. This low water intake can be the consequence of the strong odor of bioactive molecules such as allicin contained in garlic, which prevents the birds from consuming more water and feed.

Regardless of mode of administration, feeding chickens with *Allium sativum* significantly increased live weight and weight gain. This result is similar to that of Lewis et al.[30] and Al-Kassie et al.[31] who reported a positive effect of garlic on live weight and weight gain of chickens. It has also been reported in numerous studies that supplementing broilers with garlic improved the live weight [32,33]. Similarly, the addition of variable rates of garlic extract (1, 1.5, and 2.25 ml/kg feed) improves chicken live weight by up to 5.8%[34]. In contrast, Mohebbifar and Torki[34] reported that the inclusion of garlic powder in broiler chicken diets did not affect body weight, feed intake, and feed conversion. In the present study, the improved live weight and weight gain of broilers fed on garlic could be due to the action of allicin, which inhibited the growth of pathogenic bacteria by interfering with bacterial cell metabolism[36]. Garlic is also thought to improve the activity of pancreatic enzymes and activate the digestive system, a process that promotes nutrient absorption and ultimately growth[37].

Oral administration of *Allium sativum* resulted in a significant drop in the feed conversion ratio. This drop in feed conversion is the logical result of a drop in feed intake and a significant increase in weight gain observed in the chickens. This result is similar to that of Mahmood et al[38], who reported an improvement in feed conversion when a garlic grind was fed to broilers at a dose of 5g/kg of feed. However, Amouzmehr et al.[39] found no significant effect of garlic extracts at 0.6% on broiler feed conversion.

The incorporation of *Allium sativum* into the diets did not significantly affect the carcass characteristics, relative organ weights, and digestive organ measurements of broilers, regardless of the mode of administration. This result is in agreement with the findings of another study[40], indicating no significant effect with the incorporation of 0.25% of the ginger-garlic mixture in the chicken feed on carcass characteristics. Aji et al.[41] reported similar results with garlic and onion in broilers.

Oral administration of garlic induced a non-significant increase in the lactobacilli count in the digestive tract of the chicken. Work by Navidshad et al [42] revealed that the antibacterial effect of aqueous garlic extract is comparable to that of the antibiotic enrofloxacin. This result suggests that incorporating *Allium sativum* promotes the development of o. Some experiments indicate that this plant can improve the intestinal microflora of chickens. Allicin has a bactericidal effect that limits the development of pathogenic bacteria [43]. This result is in agreement with the work of Peinado et al.[12], who reported that supplementing broiler feed with garlic extract at doses of 45 and 135 ml/kg reduced the number of enteropathogens and improved the ileal mucosa and growth performance. In the same line, Ismoyowati et al.[44] reported that aqueous garlic extract has considerable growth inhibitory activity against *E. coli* and *S. pullorum*. Kumar et al [45] reported that the mixture of plant extracts (Eugenol) and garlic attenuated the effect of necrotic enteritis and improved the intestinal health of chickens. This activity is attributed to the numerous phytochemical compounds (phenols, flavonoids, and thiosulfate) present in garlic in varying concentrations and their synergistic effect[46].

Oral administration of garlic induced a significant drop in serum AST and ALT content compared with the negative control. This result is in agreement with the findings of El-Latif et al.[47] who stated that the addition of 100 and 200 mg garlic oil/kg feed significantly reduced the concentration of AST in broilers. The decrease in liver function markers in the present study remains within the normal threshold and is thought to be due to bioactive molecules such as diallyl disulfide and trisulfide present in garlic. Administration of garlic via water and feed to broilers had no significant effect on serum total protein concentration. This result is similar to the result of Onu et al.[40] who revealed that the addition of 0.25% of the ginger-garlic mixture to the broiler diets did not significantly affect serum total protein concentration. Contrary, Oleforuh-Okoleh et al.[48] reported that 50 ml of aqueous extract of the ginger-garlic mixture per liter of drinking water significantly increased serum total protein concentration in broilers, suggesting that the result depend on the mode of administration.

Garlic oral administration used as a phyto-additive via drinking water induced a significant drop in serum urea content. According to Pritchard et al.[49], the decrease in serum urea concentration (hypo-uremia) may be due to dehydration, anorexia, kidney damage, etc., but more so to advanced liver failure (cirrhosis). In the present study, this reduction was due to the dehydration of broilers induced by the incorporation of *Allium sativum* into their drinking water, which significantly reduced their water consumption. Dietary incorporation of garlic in broilers' feed resulted in a significant increase in serum creatinine content. This result contradicts Dieumou et al.[50] findings which revealed that ginger and garlic essential oils administered by gavage at doses of 10, 20, and 40 mg/kg/day had no effect on serum creatinine content in broilers. In the same line, Oleforuh-Okoleh et al.[49] reported that 50 ml of aqueous extract of the ginger-garlic mixture per liter of drinking water had no significant effect on urea concentration in broilers serum.

The inclusion of garlic in feed and drinking water induced a significant decrease in serum total cholesterol content compared to positive control. This result corroborates that of Onyimonyi et al [51] who recorded a decrease in cholesterol levels with increasing levels of garlic in the Diets. Ao et al.[52] also reported that the addition of 0.2% fermented garlic to a broiler diet, containing soybean oil as the sole fat source, significantly lowered serum total cholesterol levels. According to Issa and Omer[53] garlic powder at different concentrations induced a significant decrease in the mean value of total cholesterol. This could be due to garlic's hypocholesterolemia activity[54]. Garlic has been shown to have cholesterol-lowering and lipid-lowering properties that act on the liver[55]. This result contradicts the findings of Amouzmehr et al.[39] who reported that adding garlic at 0.3% and 0.6% to broiler diets did not affect serum cholesterol levels in broilers. This study revealed that supplementing broilers' diets with garlic had no significant effect on serum triglyceride levels regardless of the mode of administration. This result is contrary to Prasad et al.[54] and Issa and Omer [53] who reported

that garlic powder at different concentrations induced a significant decrease in serum triglyceride levels.

Supplementing broilers' diet with *Allium sativum*, has no significant effect on serum LDL-cholesterol level regardless of the mode of administration, as well as that of HDL-cholesterol through drinking water. This result is contrary to the findings of Issa and Omer[53] who reported that dietary supplementation of garlic powder at different concentrations caused a significant decrease in mean LDL-cholesterol values while serum HDL-cholesterol concentration significantly increased in chickens up to 8 weeks of age compared to the control group. Similarly, Mansoub[56] reported that supplementing chicken feed with 1g *Allium sativum*/kg reduced LDL cholesterol and triglyceride levels. Work by Rahimi et al.[57] on this same phytobiotic revealed an increase in HDL cholesterol levels. According to Choi et al.[58], supplementation of broilers diets with 5% or 3% garlic powder and a-tocopherol significantly reduces total low-density lipoprotein (LDL) cholesterol levels and increases high-density lipoprotein (HDL) cholesterol levels.

The present study revealed that oral administration of garlic had no significant effect on feed digestibility whatever the mode of administration (water or feed). However, although not significant, the digestibility of DM, OM, CP, and NDF tended to increase with the incorporation of *Allium sativum* in the drinking water. This result is similar to that of Issa and Abo Omar [53] who reported that garlic powder incorporated at rates of 0.2 or 0.4% into the diets increased feed digestibility in broilers. The trend towards improved digestibility of feed components recorded in this study could be linked to the ability of active ingredients such as phenols and flavonoids present in garlic to destroy pathogenic micro-organisms in the digestive system, resulting in an increase in the number of beneficial bacteria in the broilers gut. The active ingredients in plants and spices act on the balance of the intestinal flora by activating digestibility and stimulating the secretion of endogenous digestive enzymes, thereby improving feed digestibility and growth performance[59,60].

5. Conclusion

The result of the present work suggests that garlic compounds are well preserved in the supplemented diet and can be administered via feed (5 g/kg) or drinking water (5 g/liter) as an alternative to growth-promoting antibiotics to improve the growth performance of broilers, without adversely affecting liver and kidney function and alleviate consumer concerns about bacterial resistance and antibiotic residues in poultry products. It would be desirable to extract, isolate, and quantify the main bioactive compounds present in *Allium sativum*, and to assess their individual effects on growth performance and markers of kidney and liver function in broilers.

Declarations

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Djamen Tchantchou Chamberlin, Kana Jean Raphael, and Nyembo Kondo Camile conceived, designed the research, and wrote the manuscript. Ngouana Tadjong Ruben, Donfack Mikael, Fokam Tagne Achille Bernard collected the data, carried out data analysis, and wrote the manuscript. Tchoun Gilchrist revised the manuscript. All authors read and approved the final manuscript.

Funding

This research received no external funding.

Availability of data and materials

The datasets generated during and analyzed during the current study are available from the corresponding author upon reasonable request.

Ethical considerations

All authors have reviewed this work for ethical problems, such as plagiarism, consent for publication, misconduct, data manipulation and/or deceit, and duplication of work.

Acknowledgments

The authors are thankful to the Faculty of Agronomy and Agricultural Sciences for providing an ideal framework for data collection

References

1. Aouadi D and Ben Salem H. Effets de l'administration des huiles essentielles de Rosmarinus officinalis et d'Artemisia herba alba sur l'ingestion et la digestion chez des béliers de race 2 et 3 décembre 2020 25e *édition Congrès international francophone - Centre des Congrès Paris La Villette*. 2012. Available at: https://scirp.org/reference/referencespapers? referenceid=2284370
2. Salahshoori Niaei F, Farah Taj Navab A. Effects of *Valeriana officinalis* and Ciprofloxacin on Kidney Histopathology in Rats Pyelonephritis by *Pseudomonas aeruginosa*. Res. Biotechnol. Environ. Sci. 2022; 1(1): 23-27. DOI: 10.58803/RBES.2022.1.1.05
3. Nyembo KC, Pascaline C, Hervé T., Innocent AM, Gilchrist TD, Wilfried ENL, Romario TT, Evariste T, and Raphael KJ. 2023a. Effects of *Cyperus alternifolius, Echinochloa pyramidalis, Typha angustifolia* and *Imperata cylindrica* on growth performance, feed digestibility, gut microbiota, heamato-biochemical and immunity parameters in broiler chikens. J World's Poult Res. 2023a; 13(2): 268-279. DOI: 10.36380/jwpr.2023.30
4. Nyembo KC, Tchoffo H, Chongsi MM, Ciza AP, Amani MI, Tieubou TL, and Kana JR. 2023b. Effects of gradel levels of *Typha angustifolia* rhizomes on intestinal microflora, immune system, and growth performance of broiler chikens. J World's Poult Res. 2023b; 2(4): 39-46. DOI: 10.58803/jwps.v2i4.20
5. Edie Nounamo LW, Ngouana Tadjong R, Ciza Azine P, Ebile Dayan A, Nanken Tsague CD, Pimagha Moffo HJ, Kemmo Kenhago AP, Kana JR. Effects of in-feed blend spices on growth performance, digestibility profile and production cost of Japanese quails (*Coturnix japonica*). Sci J Anim Sci. 2022; 10(4) 747-757. DOI: 10.14196/sjas. v10i4.1710

6. Bashige V, Bakari A, Okusa P, Kalonda E, and Lumbu J. Criblage phytochimique et activité antimicrobienne de six rhizomes comestibles utilisés en médecine traditionnelle à Lubumbashi (RDC). Int J Biol Chem Sci. 2020; 14(4): 1367-1380. DOI: 10.4314/ijbcs.v14i4.16
7. Mohammad BH, Mohammad AA, Mohammad SS, and Doulati B. Essential oil composition of Zingiber officinale Rosc. Rhizome from the Iranian herb market. Chemija. 2011; 22(1): 5659. Available at: https://www.academia.edu/30413558/Essential_oil_composition_of_Zingiber_officinale_Rosc_rhizome_from_the_Iranian_herb_market
8. Goetz P, and Ghédira K. Phytothérapie anti-infectieuse, collection phytothérapie practice. Paris: Springer; 2012. Available at: https://www.eyrolles.com/Sciences/Livre/phytotherapie-anti-infectieuse-9782817800578/
9. Oleforuh-Okoleh VU, Ndofor-Foleng HM, Olorunleke SO, and Uguru JO. Evaluation of growth performance, haematological and serum biochemical response of broiler chickens to aqueous extract of ginger and garlic. J Agric Sci. 2015; 7(4): 167-173. Available at: https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=8f9c06d00b0776bfea32aba8652629c18f530518
10. Fankam AG, Kuete V, Voukeng IK, Kuiate JR, and Pages JM. Antibacterial activities of selected Cameroonian spices and their synergistic effects with Antibiotics against multidrug-resistant phenotypes. BMC Complement Altern Med. 2011; 11: 104. DOI : 10.1186/1472-6882-11-104
11. Dahiya P, and Purkayastha S. Phytochimical screening and antimicrobial activity of some medicinal plants against multi-drug resistant bacteria from clinical isolates. Indian J Pharm Sci. 2012; 74(5): 443-50. DOI: 10.4103%2F0250-474X.108420
12. Peinado MJ, Ruiz R, Echávarii A, Aranda-Olmedo I, and Rubio LA. Garlic derivative PTS-O modulates intestinal microbiota composition and improves digestibility in growing broiler chickens. Anim Feed Sci Technol. 2013; 181(1-4): 87-92. Available at: https://www.infona.pl/resource/bwmeta1.element.elsevier-4a49b11f-8e61-3902-99a7-5554eccdbf83
13. Mikaili P, Surush M, Milad M, Shahin A, and Shadi S. Therapeutic uses and pharmacological properties of garlic, shallot, and their biologically active compounds. Iran J Basic Med Sci. 2013; 16(10): 1031-1048. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3874089/
14. Rodrigues LLO, de Oliveira ACL, Tabrez S, Shakil S, Khan MI, Asghar MN, et al. Mutagenic, antioxidant and wound healing properties of Aloe vera. J Ethnopharmacol. 2018; 227: 191-197. DOI: 10.1016/j.jep.2018.08.034
15. Hu Y, Xu J, and Hu Q. Evaluation of antioxidant potential of aloe vera (Aloe barbadensis Miller) extracts. J Agric Food Chem. 2003; 51(26): 7788-7791. DOI: 10.1021/jf034255i
16. Jesus Cardenas. European Scientific Cooperative On phytoterapy (ESCOP), Directeur médical de Doctissimo, Révision Médicale. 2012. Available at : https://www.doctissimo.fr/equipe/auteurs/dr-jesus-cardenas
17. Rinkesh B, Makwana Ajay P, Raval Haresh D, Chauhan Ram C, Kulkarni Amit S, rivastava K, et al. Effects of garlic (Allium sativum) supplementation on growth performance, carcass characteristics and economics of broilers. J Anim Res. 2015; 5(4): 843-848. DOI: 10.5958/2277-940X.2015.00140.0
18. Franciszek Brzóska, Bogdan Sliwinski, Olga Michalik-Rutkowska, and Józef Sliwa. The effecT of garlic (Allium sAtivum) on growth performance, mortality rate, meat and blood parameters in broilers. Ann Anim Sci. 2015; 15(4): 961-975. DOI: 10.1515/aoas-2015-0052
19. Harris JC, Cottrell SL, Plummer L, and loyd SD. Antimicrobial properties of Allium sativum (garlic) Mini-Review. Appl Microbiol Biotechnol. 2001; 57(3): 282-286. DOI: 10.1007/s002530100722
20. Chiang Y, Jen L, Su H, Lii C, Sheen L, and Liu C. Effects of garlic oil and two of its major organosulfur compounds, diallyl disulfide and diallyl trisulfide, on intestinal damage in rats Injected with endotoxin. Toxicol Appl Pharmacol. 2006; 213(1): 46-54. Available at: https://pubmed.ncbi.nlm.nih.gov/16274720/
21. Afnor. Essais des eaux -Détermination de l'inhibition de luminescence de Photobacterium phosphoreum [Water tests - Determination of inhibition of Photobacterium phosphoreum luminescence]. 1991 ; National standards and national normative documents. Norme expérimentale NT90-320. Available at: https://www.boutique.afnor.org/fr-fr/norme/t90320/essais-des-eauxdetermination-de-linhibition-de-la-luminescence-dephotobac/fa024059/77210
22. Ngouana TR, Kana JR, Takumbo TBD, Mube KH, Yemdjie MDD, Teguia A, and Meimandipour A. Effects of dietary thyme and oregano essential oils entrapped in chitosan and Canarium charcoal stable matrix on growth performances in broiler chickens. Int J Innov Appl Stud. 2018; 22 (2), 114-122. Available at: https://search.proquest.com/openview/b030f6e008eef824bcf2ae5122f0e98c/1?pq-origsite=gscholar&cbl=2031961
23. Association of official analytical chemists (AOAC) (1990). Official methods of analysis, 15th Edition. Washington, DC, USA. Available at: https://law.resource.org/pub/us/cfr/ibr/002/aoac.methods.1.1990.pdf
24. Van Soest PJ, Robertson JB, and Lewis BA. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci. 1991; 74(10): 3583-3597. DOI: 10.3168/jds.S0022-0302(91)78551-2
25. Pourali M, Mirghelenj SA, and Kermanshahi H. Effects of garlic powder on productive performance and immune response of broiler chickens challenged with Newcastle disease virus. Glob Vet. 2010; 4(6): 616-621. Available at: http://www.idosi.org/gv/gv4(6)10/16.pdf
26. Jimoh AA, Ibitoye EB, Dabai YU, and Garba S. In vivo antimicrobial potentials of garlic against clostridium perfringens and its promotant effects on performance of broiler chickens. Pak J Biol Sci. 2013; 16(24): 1978-1984. DOI: 10.3923/pjbs.2013.1978.1984
27. ManSoub NH, and Myandoab MP (2011). The effect of different levels of thyme on performance, carcass traits and blood parameters of broilers. Ann Biol Res. 2(4): 379-385. Available at: https://www.scirp.org/(S(vtj3fa45qm1ean45vvff%20cz55))/reference/referencespapers.aspx?referenceid=2670150
28. Elagib HAA, El-Amin WIA, and Malik HEE. Effect of dietary garlic (Allium sativum) supplementation on broiler performance and blood profile. J Anim Sci Adv. 2013; 3(2): 58-64. DOI: 10.5455/JASA.20130219104029
29. Necdem TB, Raphaël KJ, Divine YM, Agwah ED, Ruben NT, Mikael D, et al. (2020) Effects of graded levels of joy perfume tree (Pent adiplandra brazzeana) powder in drinking water on growth performance, gut microbiota and haemato-biochemical parameters of broiler chickens. Open J Anim Sci. 10(3): 514-527. DOI: 10.4236/ojas.2020.103032
30. Lewis MR, Rose SP, Mackenzie AM, and Tucker LA. Effets de l'inclusion alimentaire des extraits de plantes sur les performances de croissance des poulets de chair mâles. Br Poul Sci. 2003; 44: 43-44. DOI: 10.1080/713655281
31. Al-Kassie GAM, Al-Nasrawi MAM, and Ajeena SJ. The effects of using hot red pepper as a diet supplement on some performance traits in broiler. Pak J Nutr. 2011; 10(9): 842-845. DOI: 10.3923/pjn.2011.842.845
32. Ademola SG, Farinu GO, and Babatunde GM. Serum lipid, growth and hematological parameters of broilers fed garlic, ginger and their mixtures. World J Agric Sci. 2009; 5(1): 99-104. Available at: https://idosi.org/bjps/2(2)13/2.pdf
33. Karangiya VK, Savsani HH, Patil SS, Garg DD, Murthy KS, Ribadiya NK, et al. Effect of dietary supplementation of garlic, ginger and their combination on feed intake, growth performance and economics in commercial broilers. Vet World. 2016; 9(3): 245-250. DOI: 10.14202/vetworld.2016.245-250
34. Brzóska F, Sliwinski B, Michalik-Rutkowska O, and Sliwa J. The effect of garlic (Allium sativum L.) on growth performance, mortality rate, meat and blood parameters in broilers. Ann Anim Sci. 2015; 15(4): 961. DOI: 10.1515/aoas-2015-0052
35. Mohebbifar A, and Torki M. Growth performance and humoral response of broiler chicks fed diet containing graded levels of ground date pits with a mixture of dried garlic and thyme. Global Veterinarian. 2011; 6: 389-398.
36. Ghosh S, Mehla RK, Sirohi SK, and Roy B. The effect of dietary garlic supplementation on body weight gain, feed intake, feed conversion efficiency, faecal score, faecal coliform count and feeding cost in crossbred dairy calves. Trop Anim Health Prod. 2010; 42(5): 961-968. DOI: 10.1007/s11250-009-9514-5
37. Ramakrishna RR, Platel K, and Srinivasan K (2003). In vitro influence of species and spice-active principles on digestive enzymes of rat pancreas and small intestine. Food. 47: 408-412. DOI: 10.1002/nourriture.200390091
38. Mahmood S, Mushtaq-UI-Hassan M, Alam M, and Ahmed F. Comparative

efficacy of Nigella sativa and Allium sativum as growth promoters in broilers. Int J Agric Sci. 2009; 11: 775-778.
39. Amouzmehr A, Dastar B, Nejad JG, Sung KI, Lohakare J, and Forghani F. Effect of garlic and thyme extracts on growth performance and carcass characteristics of broiler chicks J Animal sci technol. 2012; 54: 185-190.
40. Onu PN. Evaluation of two herbal spices as feed additives for finisher broilers. Biotechnol Anim Husb. 2010; 26(5-6): 383-392. DOI: 10.2298/BAH10063830
41. Aji SB, Ignatius K, Ado Y, Nuhu JB, Abdulkarim A, Al i yu U, et al. Effects of feeding onion and garlic on some performance characteristics of broiler chickens. Res J Poult Sci. 2011; 4(2): 22-27.
42. Navidshad B, Darabighane B, and Malecky M. 2018; Garic: An alternative to antibiotics in poultry production, a review. Iran J Appl Anim Sci. 8(1): 9-17. http://ijas.iaurasht.ac.ir/article_538739_10bf7af3d2370e37f6f2a07eb1988470.pdf
43. Durairaj S, Srinivasan S, and Lakshmanaperumalsamy P. In vitro antibacterial activity and stability of garlic extract at different pH and temperature. Electron J Biol. 2009; 5(1): 5-10. Available at: https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=70425fe4ac5bfbf4ac9a51c4869496331ce1dbc9
44. Ismoyowati, Indrasanti D, Mufti M, and Farjam AS. Photobiotic properties of garlic, red ginger, turmeric and kencur in growing ducks. Anim Product. 2015; 17(1): 49-55. Available at: http://animalproduction.net/index.php/JAP/article/view/484
45. Kumar A, Kheravii SK, Ionescu C, Blanchard A, Barekatain R, and Bajagai YS, et al. Microencapsulated mixture of eugenol and garlic tincture supplementation mitigates the effect of necrotic enteritis on intestinal integrity and increases goblet cells in broilers. Microorganisms. 2021; 9(7): 1451. DOI: 10.3390/microorganisms9071451
46. Alli JA, Okonko BE, Kolade IO, Kolade AF, and Nwanze JC. In vitro assessments of the effects of garlic (Allium sativum) extract on clinical isolates of Pseudomonas aeruginosa and Staphylococcus aureus. Advances Appl Sci Res. 2011; 2(4): 25-36. Available at: https://www.cabidigitallibrary.org/doi/full/10.5555/20133073424
47. El-Latif ASA, Saleh NS, Allam TS, and Ghazy EW. The effects of rosemary (Rosemarinus afficinalis) and garlic (Allium sativum) essential oils on performance, hematological, biochemical and immunological parameters of broiler chickens. Br J Poult Sci. 2013; 2(2): 16-24.
48. Oleforuh-Okoleh VU, Ndofor-Foleng HM, Olorunleke SO, and Uguru JO. Evaluation of growth performance, haematological and serum biochemical response of broiler chickens to aqueous extract of ginger and garlic. J Agric Sci. 2015; 7(4): 167-173. DOI: Available at: 10.5539/jas.v7n4p167
49. Pritchard JC, Burn CC, Barr AR, Whay HR. Haematological and serum biochemical reference values for apparently healthy working horses in Pakistan. Res Vet Sci. 2009; 87(3): 389-395. Available at: https://www.sciencedirect.com/science/article/pii/S0034528809001313
50. Dieumou FE, Teguia A, Kuiate JR, Tamokou JD, Fonge NB, and Dongmo MC. Effects of ginger (Zingiber officinale) and garlic (Allium sativum) essential oils on growth performance and gut microbial population of broiler chickens. Livest Res Rural Dev. 2009; 21(8): 131. Available at: https://lrrd.cipav.org.co/lrrd21/8/dieu 21131.htm
51. Onyimonyi AE, Chukwuma PC, and Igbokwe C. Growth and hypocholesterolemic properties of dry garlic powder (Allium sativum) on broilers. Afr J Biotechnol. 2012; 11(11): 2666-2671. DOI: 10.5897/AJB11.2877
52. Ao X, Yoo JS, Zhou TX, Wang JP, Meng QW, Yan L, et al. 2011. Effects of fermented garlic powder supplementation on growth performance, blood profiles and breast meat quality in broilers. Livestock Science, 141(1): 85-89. DOI: 10.1016/j.livsci.2011.05.002
53. Issa KJ, and Omar JA. Effect of garlic powder on performance and lipid profile of broilers. Open J Anim Sci. 2012; 2(6): 62-68. DOI: 10.4236/ojas.2012.22010
54. Hochleithner M. Biochemistries. In: Ritchie BW, Harrison GJ, Harrison LR, editors. Avian medicine: Principles and application Lake Worth, FL, Wingers Publishing; 1994. p. 223-245.
55. Qureshi AA, Din ZZ, Abuimeileh N, Burger WC, Ahmad Y, and Elson CE. Suppression of avian hepatic lipid metabolism by solvent extracts of garlic: Impact on serum lipids. J Nutr. 1983; 113(9): 1746-1755. DOI: DOI : 10.1093/jn/113.9.1746
56. Prasad R, Rose MK, Virmani M, Garg SL, and Puri JP. Lipid profile of chicken (Gallus domesticus) in response to dietary supplementation of garlic (Allium sativum). Int J Poult Sci. 2009; 8(3): 270-276. Available at: https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=42055ef1535d1d0f69f618800540bb11ef746b02
57. Mansoub NH, and Nezhady MAM. The effect of using Thyme, Garlic and Nettle on performance, carcass quality and blood parameters. Ann Biol Res. 2011; 2: 315-320. Available at: http://scholarsresearchlibrary.com/ABR-vol2-iss4/ABR-2011-2-4-315-320.pdf
58. Rahimi S, Teymouri ZZ, Karimi TM, Omidbaigi R, and Rokni H. Effet des trois extraits de plantes sur les performances de croissance, le système immunitaire, les facteurs sanguins et la population bactérienne intestinale sélectionnée chez les poulets de chair. 2011.
59. Choi IH, Park WY, and Kim YJ. Effects of dietary garlic powder and a-tocopherol supplementation on performance, serum cholesterol levels, and meat quality of chicken. Poult Sci. 2010; 89(8): 1724-1731. DOI: 10.3382/ps.2009-00052
60. Williams P, and Losa R. The use of essential oil and their compounds in poultry nutrition. World Poult. 2001; 17: 14-15.
61. Cross DE, McDevitt RM, Hillman K, and Acamovic T. L'effet des herbes et de leurs huiles essentielles associées sur la performance, diététique, la digestibilité et la microflore intestinale chez les poulets de 7 à 28 jours d'âge. Br Poult Sci. 2007; 48(4): 496-506. DOI: 10.1080/00071660701463221